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Molecular Studies on Bromovirus Capsid Protein
IV. Coat Protein Exchanges between Brome Mosaic and Cowpea Chlorotic Mottle Viruses Exhibit Neutral Effects in Heterologous Hosts

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Two members of the bromovirus group, brome mosaic virus (BMV) and cowpea chlorotic mottle virus (CCMV), selectively infect barley and cowpea, respectively, and also differ in their ability to systemically infect a common permissive host, *Chenopodium quinoa*. CCMV is confined to inoculated leaves of *C. quinoa*, whereas BMV causes rapid systemic mottling. To examine whether host-specific determinants for systemic movement of BMV and CCMV in each of these hosts are localized in the coat protein (CP), sequences encoding this gene were exchanged between biologically active clones of BMV RNA3 (B3) and CCMV RNA3 (C3) to create chimera expressing heterologous CP genes (B3/CCP and C3/BCP). Inoculation of each chimera with its respective wild-type (wt) RNAs 1 and 2 to barley or cowpea or *C. quinoa* plants resulted in symptom phenotype and long distance movement characteristics similar to those of the parental virus donating RNAs 1 and 2. These observations suggest that neither BMV CP nor CCMV CP has host-specific determinants for long distance movement. Inoculation of additional recombinant viruses, constructed by reassorting wt genomic RNAs 1 and 2 of BMV and CCMV with either heterologous wt RNA3 (i.e., B1+B2+C3 and C1+C2+B3) or heterologous chimeric RNA3 (i.e., B1+B2+C3/BCP and C1+C2+B3/CCP), to susceptible hosts resulted only in localized infections. The significance of these observations in relation to bromovirus movement is discussed.

INTRODUCTION

Bromoviruses are a group of icosahedral, multipartite, positive-strand RNA viruses of plants (Ahlquist, 1994). The 8-kb genome of brome mosaic virus (BMV) and cowpea chlorotic mottle virus (CCMV) is divided among three RNA components. Viral replication is dependent on two nonstructural proteins, 1a and 2a, encoded by monocistronic RNAs 1 and 2, respectively (Ahlquist, 1994; Allison *et al.,* 1988). The two gene products encoded by the dicistronic RNA3 are dispensable for viral replication but are required for systemic infection in plants (Allison *et al.,* 1990; Rao and Grantham, 1995b). The 5′-proximal gene of RNA3 encodes a nonstructural protein of 32 kDa, the designated movement protein (MP), while the coat protein (CP) is translated from a subgenomic RNA derived *in vivo* from progeny minus RNA3 by internal initiation (Miller *et al.,* 1985). BMV has a narrow host range and mostly infects monocotyledonous plants such as barley (Ahlquist, 1994). By contrast, CCMV infects a broad range of dicotyledonous plants, and cowpea has been the experimental host. Despite this host specificity, several *Chenopodium* spp. and *Nicotiana benthamiana* have been the common permissive hosts for BMV and CCMV (Mise *et al.,* 1993; Rao, 1997; Rao and Grantham, 1995a).

The division of function among the three genomic RNAs of bromoviruses is amenable to characterizing specific functions dictated by each gene product either individually or collectively in virus–host interactions (Allison *et al.,* 1988; Bancroft, 1972; Mise and Ahlquist, 1995; Rao and Grantham, 1995b, 1996). Genetic analyses of chimeric viruses involving the exchange of MP between BMV and CCMV revealed that this gene is critical for bromovirus host specificity (Mise and Ahlquist, 1995). Although the CPs of BMV and CCMV have been shown to be essential for systemic infection (Allison *et al.,* 1990; Rao and Grantham, 1995b, 1996; Sacher and Ahlquist, 1989), it is unknown whether the determinants distributed on the CP genes that modulate viral movement are host specific. To examine the extent to which the CPs of BMV and CCMV interact with their hosts to control symptom expression and promote movement, we have constructed RNA3 chimera by exchanging the CP genes between these two bromoviruses. This study reports the effect of heterologous CP genes on pathogenicity and movement characteristics in several host plants when coinoculated with either homologous or heterologous parental wild-type (wt) RNAs 1 and 2.

MATERIALS AND METHODS

BMV and CCMV clones used in this study

Full-length cDNA clones corresponding to the three genomic RNAs of BMV, pT7B1, pT7B2, and pT7B3, from

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which wt infectious RNAs 1 (B1), 2 (B2), and 3 (B3), respectively, can be transcribed *in vitro* have been described previously (Dreher *et al.,* 1989). Similarly, plasmids pCC1TP1, pCC2TP2, and pCC3TP4 are wt cDNA clones of CCMV RNAs 1 (C1), 2 (C2), and 3 (C3), respectively (Allison *et al.,* 1988).

Construction of BMV and CCMV RNA3 chimera bearing heterologous CP genes

BMV and CCMV CP genes (hereafter designated BCP and CCP, respectively) were exchanged between cDNA clones of respective RNA3s as follows: The entire CCP gene sequence was amplified from pCC3TP4 in a polymerase chain reaction (Rao and Grantham, 1996) using a 5′ oligonucleotide primer (dTTTATCATGTCGACAGTCGGA; underlined bases contain a *Sal*I restriction site) and a 3′ oligonucleotide primer (dCTTCAGCGAGGCCTAATACACC; underlined bases contain a *Stu*I restriction site) and the resulting product was digested with *Sal*I and *Stu*I and subcloned into similarly treated pT7B3 to yield pT7B3/CCP. To replace CCP with BCP in pCC3TP4 clone, a PCR product encompassing the BCP gene sequence was amplified with a 5′ oligonucleotide primer (dGGAACTGGTAAGTTAACTCGCGCGCAG; *Hpa*I site is underlined) and a 3′ oligonucleotide primer (dGGGGCTCTCCGACTAGTGGCACTACCT; *Spe*I site is underlined) and the resulting PCR product was digested with *Hpa*I and *Spe*I and subcloned into similarly treated pCCTP4, to yield pCC3/BCP. The sequences of the subcloned fragments were confirmed by restriction digest mapping and sequencing (Rao and Grantham, 1996).

In vitro transcription and transfection of protoplasts

Prior to transcription, all three BMV wt clones and the pT7B3/CCP clone were linearized with *Bam*HI (Dreher *et al.,* 1989), whereas all three CCMV wt clones were linearized with *Xba*I (Allison *et al.,* 1988). pCC3/BCP was linearized with *Sal*I because of the presence of an internal *Xba*I site in the BCP coding region. Capped full-length transcripts were synthesized *in vitro* using a MEGAscript T7 kit (Ambion, Inc., Austin, TX). Protoplasts were prepared from 6-day-old barley plants (*Hordeum vulgare* L. cv. Dickson) and transfected with the desired combination of *in vitro* transcripts using polyethylene glycol (Rao *et al.,* 1994).

RNA isolation and Northern blot analysis

The procedures used to extract progeny RNA from transfected protoplasts and their analysis by Northern hybridization using riboprobes of desired specificity were performed as described previously (Rao *et al.,* 1994; Rao and Grantham, 1995b). The synthesis of ^{32}P-labeled riboprobes to detect positive-strand BMV RNAs has been described previously (Rao *et al.,* 1989). A T3 RNA polymerase transcript from pT7T3CC3t, constructed by cloning a blunt-ended *Spe*I–*Sal*I fragment (bases 1953–2173) corresponding to the 3′ noncoding region of pCC3TP4 into the *Sma*I site of pT7T3-18U (Pharmacia), was used to detect progeny (+)-strand sequences of all four CCMV RNAs. A T7 RNA polymerase transcript from pT7T3B3CB, constructed by cloning a *Cla*I–*Bgl*II fragment (bases 601–1221) of pT7B3 into the *Bam*HI–*Acc*I site of pT7T3-18U, was used to detect (+)-strand BMV MP sequences. A T3 RNA polymerase transcript of pT7T3CCMP, constructed by cloning a blunt-ended *Bgl*II fragment (bases 430–1150) of pCC3TP4 into the *Sma*I site of pT7T3-18U, was used to detect (+)-strand CCMV MP sequences.

Biological assays

Barley (*H. vulgare* cv. Dickson), *Chenopodium quinoa,* and cowpea (*Vigna sinensis* cv. Blackeye) plants were kept in the dark for at least 18 h and dusted with Carborundum prior to inoculation. They were mechanically inoculated with 10 μl/leaf of a mixture containing viral RNA transcripts in desired combinations (150 μg/ml; Rao *et al.,* 1994). Each experiment was repeated at least three times with independently synthesized *in vitro* transcript preparations. The inoculated plants were kept in the greenhouse at 25° and observed for symptom expression over a period of 2–3 weeks. Absence of visible symptoms was not considered to be conclusive evidence for the noninfectious nature of the inoculum; therefore total RNA isolated from asymptomatic leaves was analyzed by Northern blots.

Western blot analysis

Proteins for Western blot analysis were obtained from either infected leaf tissue or transfected protoplasts (2.5×10^5). Samples were suspended in SDS–PAGE sample buffer (final concentration: 125 mM Tris, pH 6.8, 10% [w/v] glycerol, 2.5% [w/v] dithiothretol, 2% SDS, 0.01% bromophenol blue), denatured at 100° for 5 min, and fractionated on a 14% SDS–PAGE according to Laemmli (1970). Fractionated proteins were electrophoretically transferred to a nitrocellulose membrane and detected with either BMV CP antibody (1:1000 dilution) or CCMV CP antibody (1:800 dilution) and goat anti-rabbit secondary antibody conjugated with alkaline phosphatase (Bio-Rad).

RESULTS

Characteristic features of the CPs of BMV and CCMV

BCP and CCP are composed of 189 and 190 amino acids, respectively, and share 70% identity at the amino acid level (Speir *et al.,* 1995). Because of this extensive homology, the respective CP antisera exhibited cross-reactivity in Western blots (Fig. 2B) but not in agar gel immunodiffusion tests (Bancroft, 1972). Although the number of amino acid residues in CCP differs from that of BCP by only 1 amino acid, the distinctive electrophoretic mobility exhibited by each CP, perhaps due to the confor-

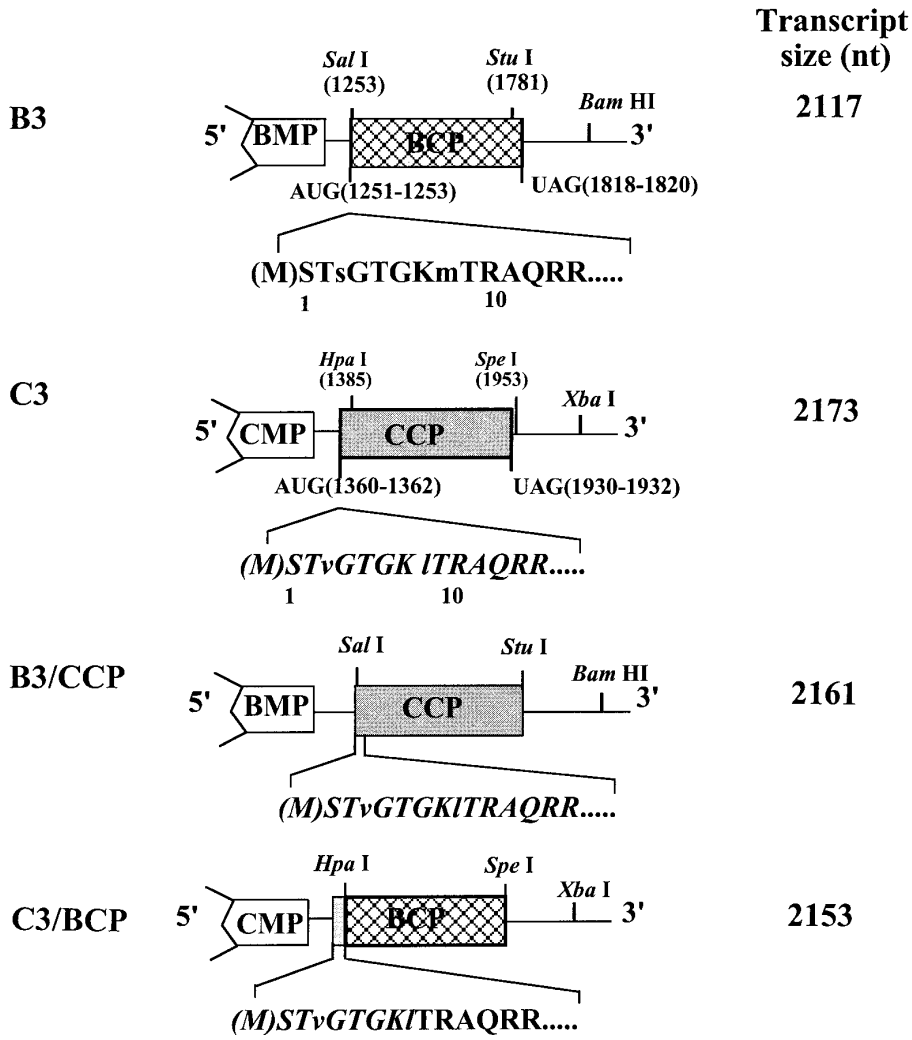

FIG. 1. Characteristics of BMV and CCMV CP chimera. The structures of wt BMV RNA3 (B3) and CCMV RNA3 (C3) are shown with noncoding sequences represented as single lines and a portion of BMV movement protein (BMP) and CCMV MP (CMP) as an open box. The crosshatched and stippled boxes represent BMV CP (BCP) and CCMV CP (CCP), respectively. The positions of start (AUG) and stop (UAG) codons of each CP gene are shown. Selected restriction sites used to exchange CP genes between B3 and C3 are shown. The sequences of the first 14 amino acids within the highly conserved N-terminal basic region of BCP and CCP are shown in uppercase and italicized uppercase, respectively. In bromoviruses, the initiating methionine (parentheses) is removed and the resultant N-terminal serine is acetylated in the mature CP (Moosic *et al.,* 1983). The first N-terminal 14-amino-acid region of BCP differs from that of CCP in two positions (shown in lowercase). Sizes of the *in vitro* transcripts expected from wt RNA3 (B3 and C3) and the two CP chimera (B3/CCP and C3/BCP) are shown at right.

mational differences manifested by nonconserved amino acids, is diagnostic for these two viruses (see Fig. 2B).

Characteristics and replication competence of B3/CCP and C3/BCP chimera

In wt B3, the CP open reading frame (ORF) starts at position 1251 (^{1251}AUG^{1253}) and terminates at 1820 (^{1818}UAG^{1820}; Fig. 1). A unique *Sal*I site is located at position 1253 and a *Stu*I site at position 1781 (12 amino acids upstream of the CP termination codon; Fig. 1). To facilitate easy replacement of the BCP sequence with that of CCP, cloning required engineering of *Sal*I and *Stu*I sites, respectively, at the beginning and the end of the CCP gene. RNA transcripts derived from pT7B3/CCP are designated B3/CCP and are 44 nucleotides longer than those of the parental wt B3 (Fig. 1). It was anticipated that CP subgenomic mRNA4 produced *in vivo* from (−)-strand B3/CCP progeny would translate to yield mature wt CCMV CP. Unlike BCP ORF, no unique restriction site was present at the beginning of CCP ORF, but a unique *Hpa*I site is located at position 1385 (8 amino acids downstream of the start codon; Fig. 1). Since the first N-proximal 25 amino acids of BCP and CCP are highly conserved (Fig. 1; Rao and Grantham, 1996; Sacher and Ahlquist, 1989), a *Hpa*I site was engineered at the analogous position in the BCP ORF, and the CP was subcloned into pCC3TP4 as a *Hpa*I–*Spe*I cassette (Fig. 1). As a result, RNA transcripts of C3/BCP, derived from pCC3/BCP, were 20 nt shorter than those of the parental wt C3. Thus,

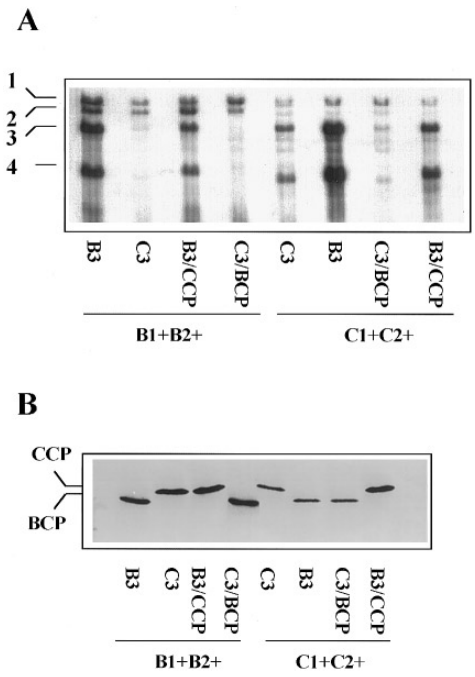

FIG. 2. Progeny analysis of BMV and CCMV CP chimera in barley protoplasts. (A) Northern analysis of replicative competence of CP chimera in barley protoplasts. Protoplasts were coinoculated with *in vitro* transcripts of either wt B1 and B2 or wt C1 and C2 and the following: wt B3, wt C3, B3/CCP, and C3/BCP. Transfected protoplasts were incubated for 48 h and RNA was extracted from protoplasts according to Rao *et al.* (1994). RNAs were analyzed on 1% agarose gels after denaturation with glyoxal and transferred to nylon membranes. Blots were hybridized with a mixture of ^{32}P-labeled (−)-sense RNA probes representing the homologous 3′ region present on each of the four BMV and CCMV RNAs. The positions of the four wt BMV RNAs are shown to the left. (B) Western blot analysis of CP accumulation. Polyacrylamide gel electrophoresis of CP from barley protoplasts coinoculated with transcripts of either wt B1 and B2 or wt C1 and C2 and the indicated RNA3 transcripts. Protoplasts were suspended in SDS–PAGE sample buffer, denatured by boiling for 5 min, and subjected to 14% SDS–PAGE. After transferring the proteins to a nitrocellulose membrane, blots were probed with anti-CCMV CP antibodies. The positions of BMV CP (BCP) and CCMV CP (CCP) are shown to the left.

during *in vivo* replication, the first 8 N-terminal amino acids of the matured BCP translated from the subgenomic mRNA4 of C3/BCP are of CCP origin (Fig. 1). When coinoculated with respective parental RNAs 1 and 2 to barley protoplasts, B3/CCP and C3/BCP replicated and produced subgenomic mRNA4 (Fig. 2A) and translated CP of expected parental origin, as evidenced by the differences in electrophoretic mobility (Fig. 2B). While analyzing the replicative competence of two reassortants constructed by exchanging RNA3 between BMV and CCMV, Allison *et al.* (1988) observed that C3 was not replicated as well as B3 by B1+B2, while B3 was replicated better than C3 by C1+C2. We also observed similar replication profiles when progeny RNA isolated from protoplasts transfected with B1+B2+B3/CCP, B1+B2+C3/BCP, C1+C2+C3/BCP, and C1+C2+B3/CCP were subjected to Northern analysis (Fig. 2A). Although the accumulated levels of progeny RNAs 3 and 4 resulted from inocula containing B1+B2+C3 and B1+B2+C3/BCP were reproducibly lower (Fig. 2A), their presence was confirmed by prolonged exposure of the Northern blot (data not shown) and also by Western analysis (Fig. 2B).

Neither BCP nor CCP has host-specific determinants for long distance movement

Although CP is dispensable for the replication of BMV and CCMV (Allison *et al.,* 1988; French *et al.,* 1986; Rao and Hall, 1990; Schmitz and Rao, 1996), it is required to establish systemic infection in plants (Allison *et al.,* 1990; Rao and Grantham, 1995b, 1996). To understand to what extent the CPs of BMV and CCMV are involved during long distance movement in their selective hosts, *in vitro* transcripts of B3/CCP and C3/BCP were mixed with their respective parental genomic RNAs 1 and 2 (i.e., B1+B2+B3/CCP and C1+C2+C3/BCP) and inoculated to several barley and cowpea plants, the respective selective hosts for BMV and CCMV. A mixture containing all three wt transcripts of each virus served as a positive control. The phenotypic symptoms induced by wt and chimeric RNA3 in these hosts are summarized in Table 1. Control plants inoculated with all three wt genomic RNAs produced characteristic systemic infections in their respective natural hosts (Table 1) and CP was readily detected in inoculated and noninoculated upper leaves of each plant host by Western blot analysis (Fig. 3). When cowpea plants were inoculated with wt BMV and analyzed by Western blots, CP characteristic of BMV was consistently detected in inoculated, but not in noninocu-

TABLE 1

Symptoms Induced by Wild Type, RNA3 CP Chimera, and Reassortants of BMV and CCMV

Inoculum[a]	Barley systemic	Cowpea systemic	*C. quinoa* Local	*C. quinoa* Systemic
(A) wt BMV and CP chimera				
B1+B2+B3 (wt)	M	NI	CLL	SM
B1+B2+B3/CCP	M	NI	CLL	SM
(B) wt CCMV and CP chimera				
C1+C2+C3 (wt)	NI	ChM	SNLL	NI
C1+C2+C3/BCP	NI	ChM	SNLL	NI
(C) Reassortants				
B1+B2+C3/BCP	NI	NI	SCLL	NI
B1+B2+C3 (BBC)	NI	NI	NLL	NI
C1+C2+B3/CCP	NI	NI	SLI[b]	NI
C1+C2+B3 (CCB)	NI	NI	SLI[b]	NI

Note. M, mosaic; CLL, chlorotic local lesions; SCLL, small chlorotic local lesions; SM, systemic mottling; NLL, necrotic local lesions; SNLL, small necrotic local lesions; ChM, chlorotic mottle; NI, not infected.

[a] Each inoculum (150 μg/ml) contained indicated mixture of *in vitro* transcripts.

[b] SLI, symptomless infection. Although no local lesions appeared on these inoculated leaves, CP was detected in Western blot (Fig. 4B).

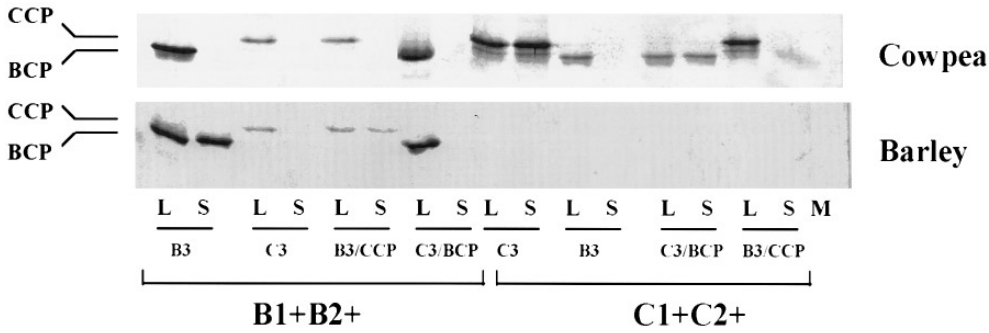

FIG. 3. Western blot analysis of CP accumulation in cowpea and barley plants. Polyacrylamide gel electrophoresis of CP from local (L) and systemic (S) leaves of cowpea and barley coinoculated with either B1+B2 or C1+C2 and the indicated wt RNA3 or CP chimera. Detection of CP is as described for Fig. 2. The positions of BMV CP (BCP) and CCMV CP (CCP) are shown to the left.

lated leaves (Fig. 3). This is probably due to limited spread, since host specificity in bromoviruses occurs after transient spread in nonhosts (Mise and Ahlquist, 1995). For the same reason, we anticipated that CCMV would also be detected in the inoculated leaves of barley. But no CP bands characteristic of wt CCMV were observed in either inoculated or noninoculated upper leaves of barley plants (Fig. 3). These results are in agreement with those of Mise *et al.* (1993).

When barley and cowpea plants were inoculated respectively with B1+B2+B3/CCP and C1+C2+C3/BCP, the symptom phenotypes induced on noninoculated upper leaves of each host plant were similar to those elicited by the parental virus providing genomic RNAs 1 and 2 (Table 1). The time of symptom appearance on these plants paralleled that of those infected with wt control inocula. To further confirm that the observed symptom phenotypes are the manifestation of infections resulting from input chimera and not due to any contaminating wt virus, CP samples recovered from each symptomatic plant were analyzed by Western blots. The diagnostic electrophoretic mobilities of BCP and CCP (Fig. 3) confirmed that the observed symptoms in each host plant are truly elicited by the inoculated chimera and not due to contamination with wt virus. The data presented above indicate that neither BCP nor CCP has host-specific determinants for long distance movement.

The role of a given viral gene in long distance movement can vary with the host plant in which it is tested (Scholthof *et al.,* 1995) and different plant species have evolved to recognize the same viral gene in different ways (Dawson, 1992). Therefore, it was envisioned that examination of the chimera in other hosts, specifically common permissive hosts with distinguishable symptom phenotypes and movement characteristics, is valuable in assessing the function of BCP and CCP in virus–host interactions. To test this possibility, we extended our infectivity assays to *C. quinoa,* a common permissive host for BMV and CCMV with distinguishable phenotypes (Table 1). In this host, by 3–4 days postinoculation (dpi) wt BMV induces characteristic chlorotic local lesions which expand with time, yielding large chlorotic blotches (Rao and Grantham, 1995b). By 8 dpi, noninoculated upper leaves display systemic mottling and downward curling (Table 1). In contrast, wt CCMV induces small chlorotic local lesions with necrotic centers and the infection is restricted to inoculated leaves only (Table 1).

C. quinoa plants were coinoculated with transcripts of B3/CCP and C3/BCP and their respective wt parental genomic RNAs 1 and 2. Parallel inoculations were also made with control inocula containing all three wt transcripts of respective parental viruses. The symptom phenotypes induced in *C. quinoa* by wt and each chimera are summarized in Table 1. As observed for the respective natural hosts (Table 1), symptom phenotypes and movement characteristics exhibited in *C. quinoa* by either B3/CCP or C3/BCP were indistinguishable from those of the parent providing wt genomic RNAs 1 and 2 (Table 1). All *C. quinoa* plants inoculated with B1+B2+B3/CCP displayed systemic mottling on a time scale similar to control inoculations made with all three wt BMV transcripts. None of the *C. quinoa* plants inoculated with C1+C2+C3/BCP developed systemic symptoms throughout the examination period, although they displayed chlorotic local lesions with necrotic centers characteristic of wt CCMV infection (Table 1). These observations were consistently recorded in at least three independent experimental trials. To further validate that observed host reactions in *C. quinoa* truly resulted from inocula containing either B1+B2+B3/CCP or C1+C2+C3/BCP, the progeny from the symptomatic leaves were subjected to a series of Northern and Western blot analyses (Fig. 4). Total RNA isolated from inoculated and noninoculated upper leaves was subjected to multiple Northern blots and hybridized with probes specific for either BMV MP or CCMV MP. As shown in Figs. 4A and 4B, in each case, Northern and Western blot analyses confirmed the chimeric nature of the recovered progeny.

Pathogenicity and movement characteristics of reassortants

Analysis of the biological properties of the two inocula, B1+B2+B3/CCP and C1+C2+C3/BCP, demonstrated that, irrespective of the host plant, neither CP has host-

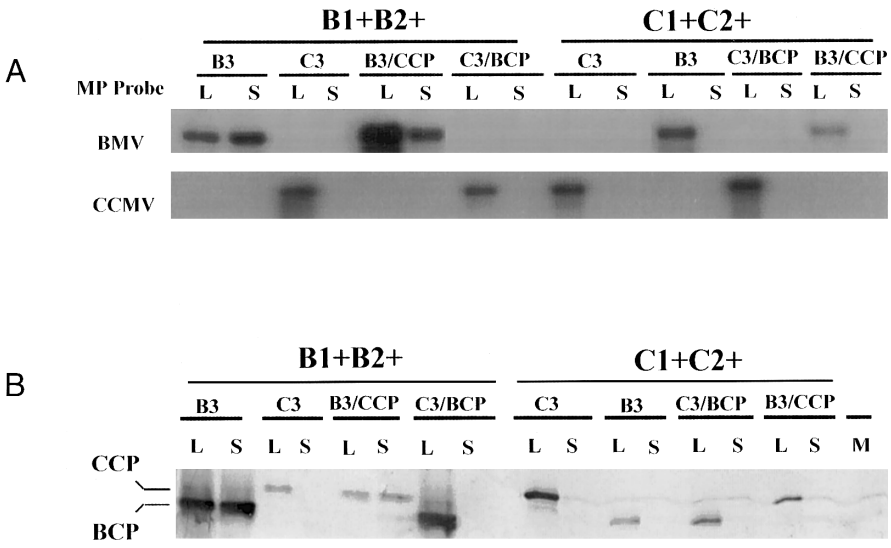

FIG. 4. Progeny analysis in *C. quinoa.* (A) Northern blot analysis of progeny RNA recovered from either local (L) or systemic (S) leaves of *C. quinoa* following inoculation with either wt B1 and B2 or wt C1 and C2 and the indicated wt RNA3 or CP chimera. Northern blot hybridization was performed as described for Fig. 2. The blots were probed with riboprobes specific for either BMV MP or CCMV MP sequences. (B) Western blot analysis of CP accumulation in either local or systemic leaves of *C. quinoa* following coinoculation with either wt B1 and B2 or wt C1 and C2 and the indicated wt RNA3 or CP chimera.

specific determinants that influence either symptom phenotype or long distance spread (Table 1). These observations imply that determinants present on genes other than the CP, i.e., either the replicase gene or the MP, independently or together, are likely to control the virus–host interactions sought in this study. To further examine this possibility, the two RNA3 chimera (i.e., B3/CCP and C3/BCP) and the wt RNA3 of each parent (i.e., B3 and C3) were heterologously reassorted with either wt B1+B2 or wt C1+C2, yielding the following four inocula: (a) B1+B2+C3/BCP, in which all but the MP are of BMV origin; (b) B1+B2+C3 (designated BBC), in which MP and CP are of CCMV origin; (c) C1+C2+B3/CCP, in which all but MP are of CCMV origin; and (d) C1+C2+B3 (designated CCB), in which MP and CP are of BMV origin. Transfection of all four inocula to barley protoplasts resulted in replication (Fig. 2A) and synthesized CP of expected parental origin (Fig. 2B).

All four inocula were independently inoculated to barley, cowpea, and *C. quinoa* plants and progeny were subjected to Northern and Western blot analysis. Results are summarized in Table 1. Irrespective of the reassortment, none of the four inocula induced any visible symptoms on noninoculated upper leaves of either barley or cowpea (Table 1). Western blot analysis failed to detect CP in noninoculated upper leaves of these hosts (Fig. 3), although the CPs with expected electrophoretic mobility were detected in the inoculated leaves of barley for B1+B2+C3/BCP and BBC (Fig. 3) and of cowpea for all four inocula (Fig. 3). By contrast, inoculation of *C. quinoa* with each of the four inocula resulted in discernable reactions (Table 1). Two of the four inocula, B1+B2+C3/BCP and BBC induced visible local lesions on the inoculated leaves of *C. quinoa* (Table 1). In both cases, the onset of lesion appearance was delayed by 3 days and the infection confined to inoculated leaves. Northern blot analysis of progeny RNA hybridized with MP-specific probes (Fig. 4A) and Western blot analysis of CP (Fig. 4B) confirmed that the progeny maintained the chimeric nature of each RNA3 present in the input inoculm, and therefore it was concluded that the observed lesions were not due to contaminating wt virus. None of the noninoculated upper leaves of these plants displayed systemic symptoms (Table 1) and no progeny could be detected in either Northern and Western blot assays (Figs. 4A and 4B). No visible local lesions appeared on the inoculated leaves of *C. quinoa* with either C1+C2+B3/CCP or CCB (Table 1). However, in each case, progeny RNA and CP were detected by Northern (Fig. 4A) and Western analysis (Fig. 4B), respectively. Noninoculated upper leaves of these plants remained symptomless (Table 1) and no progeny could be recovered (Figs. 4A and 4B).

DISCUSSION

The major objective of this study is to examine whether CP-mediated long distance movement in bromoviruses is regulated in a host-specific manner. This was tested by analyzing the biological activity of two RNA3 chimera in which the CP genes were exchanged between monocot-adapted BMV and dicot-adapted CCMV. Infectivity and host range assays indicate that CP can be freely exchanged between the two viruses without having a detectable effect on long distance movement in hosts selective for each parental virus. This was exemplified by the systemic spread observed for B1+B2+B3/CCP in barley and C1+C2+C3/BCP (having the first eight N-

terminal amino acids of CCMV CP) in cowpea (Table 1). Likewise, despite having CCMV CP, B1+B2+B3/CCP was also able to move systemically in *C. quinoa,* a feature inherent to BMV (Rao and Grantham, 1995b, 1996; Table 1). Taken together, our observations suggest that neither CP harbors a host-specific determinant for long distance spread. In contrast to these observations, viral CP functioning as a host-specific determinant for long distance movement was observed for tobamo and cucumoviruses. For example, in the case of tobacco mosaic virus (TMV) and its close relative, odontoglossom ringspot virus (ORSV), CP regulates in part the ability of TMV or the inability of ORSV to move long distances in tobacco (Hilf and Dawson, 1993). Similarly, the ability of cucumber mosaic cucumovirus (CMV) and inability of tomato aspermy cucumovirus to move long distances in cucumber is specified by the CP (Salanki *et al.,* 1997; Taliansky and Garcia-Arenal, 1995).

Having established that neither BMV nor CCMV CP has host-specific determinants to mediate long distance spread, we examined to what extent the viral replicase and MP genes are involved in this active process (Traynor *et al.,* 1991) by inoculating barley, cowpea, and *C. quinoa* plants with four hybrid viruses, BBC, CCB, B1+B2+C3/BCP, and C1+C2+B3/CCP (Table 1). Since none of the hybrid viruses were competent for systemic infection in these hosts (Table 1), the inability of the four hybrid viruses to infect their respective natural and heterologous hosts may have been the result of two independent interactions. For example, (i) the inability of B1+B2+C3/BCP and BBC to infect barley is due to MP incompatibility with the host (Mise *et al.,* 1993) and (ii) the inability these two reassortants to infect cowpea is due to replicase incompatibility with the *cis*-acting sequences on RNA3 (Pacha and Ahlquist, 1991). Our observations that only inocula containing replicase and MP from the same parent were able to establish a systemic infection in the selective host of that parent, as observed for B1+B2+B3/CCP in barley and C1+C2+C3/BCP in cowpea (Table 1), are in agreement with those reported for genetically related cucumoviruses (Salanki *et al.,* 1997). By contrast, De Jong and Ahlquist (1992) demonstrated that a movement protein hybrid constructed between CCMV and sunhemp mosaic tobamovirus (SHMV) is competent for systemic infection in cowpea. Although the reasons for the discrepancy are currently unknown, we wish to offer the following explanation. In all our experiments, each inoculum containing a mixture of heterologous RNAs in desired combination (BBC, CCB, B1+B2+C3/BCP, and C1+C2+B3/CCP; Table 1) was tested at 150 μg/ml concentration (i.e., 1 μg of each RNA transcript in 20 μl and 10 μl/leaf were used), whereas Allison *et al.* (1988) tested their two reassortants (CCB and BBC) at a concentration of 480–960 μg/ml. None of the inocula examined either by Allison *et al.* (1988) or in this study (Table 1) were able to establish systemic infection in either cowpea or barley (Allison *et al.,* 1988; Table) or in *C. quinoa* (Table 1). By contrast, De Jong *et al.* (1997) used a high concentration of inoculum (40 μg of each transcript in 50 μl, i.e., 2.4 mg/ml) to infect cowpeas with the CCMV–SHMV hybrid. Although CCMV produces more movement protein than it normally requires to establish systemic infection in cowpea (De Jong *et al.,* 1997), the rationale for using high inoculum dose is not obvious. Therefore, the systemic infection seen by De Jong *et al.* (1997) for CCMV–SHMV hybrid in cowpea could be due to application of a high inoculum level that might have resulted in overcoming the resistance of the host.

In addition to the lack of proper interaction between viral gene products, the inability of the two reassortants, BBC and CCB, constructed by Allison *et al.* (1988), as well as those examined in this study (Table 1) to systemically infect the natural and heterologous hosts might also be due to direct or indirect contribution of RNA sequences to the movement process. For example, Allison *et al.* (1988) observed that C3 was not replicated as well as B3 by B1+B2 while B3, however, was a better template than C3 for C1+C2. In this study, we also recorded similar effects not only with B3 or C3 but also with their CP chimera (Fig. 2A). Subsequently, Pacha and Ahlquist (1991) showed that this differential amplification was due to the improper adaptation of bromovirus replicase genes to RNA3 noncoding sequences. Taken together, these observations suggest that the replicase needs to be adapted to the noncoding sequences of RNA3 and absence of proper adaptation between *cis*- and *trans*-acting replication factors could significantly influence virus replication and gene expression, which subsequently prevent systemic infection.

Another important factor that affects viral movement is host resistance due to incompatible interaction between host components and viral gene products (Carrington *et al.,* 1996; Rao, 1997). For example, we recently demonstrated that CCMV can move between cells without CP, whereas BMV cannot (Rao, 1997). In this study, two reassortments B1+B2+B3/CCP and C1+C2+C3/BCP, despite having heterologous CP genes, are fully competent for systemic infection in barley and cowpea, respectively. A likely possibility for the defective cell-to-cell movement observed in our previous studies for several BMV CP-defective variants (Schmitz and Rao, 1996; Rao, 1997) is perhaps due to the inability of the virus to overcome host resistance in the absence of a functional CP, as previously surmised by Rao and Grantham (1995b, 1996). Finally, the reduced rate of replication exhibited by the reassortants examined in this study as well as by Allison *et al.* (1988) could also have influenced systemic spread, since a systemic infection can only occur if the rate of viral replication and movement can successfully keep up with the rate of plant growth (Dawson and Hilf, 1992).

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